ALLOSTERIC REGULATION OF THE ACTIVITY OF CITRATE SYNTHETASE OF ESCHERICHIA COLI BY α-KETOGLUTARATE[1]

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Various workers have demonstrated that the tricarboxylic acid cycle in some microorganisms can be divided operationally into three units, each unit being controlled independently of one another (Gray, Wimpenny and Mossman, 1966; Amarsingham and Davis, 1965, Hanson and Cox, 1967). The first unit consists of the sequence of reactions leading from citrate to α-ketoglutarate. The latter intermediate is not only used for purposes of energy generation, but also for the biosynthesis of the glutamate family of amino acids (Kornberg, 1963). In accordance with the well known principles of biological feed-back (See, review, Stadtman, 1966), the first enzyme of this pathway in E.coli, citrate synthetase, is inhibited (Weitzman, 1966) by $NADH_2$ (a potential source of energy). We show in the present communication that α-ketoglutarate also serves as an allosteric inhibitor of this enzyme.

EXPERIMENTAL PROCEDURE

E. coli B cells, grown in mineral salts medium with 0.4% acetate as the sole carbon source, were harvested in the

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stationary phase of growth and broken by sonication. Citrate synthetase was purified by the method of Weitzman (1967) to a specific activity of 30-35 umoles of coenzyme A produced/min/mg of protein (about 30-fold purified). The enzyme activity was measured according to Srere, Brazil and Gonen (1963). The assay mixture consisted of 0.02 M Tris-HCl, pH 8.0, 0.1 mM oxalacetate, 0.04 mM acetylCoA, 0.025 mM 5.5'-dithiobis-(2-nitrobenzoic acid), and a suitable concentration of the enzyme. The reaction was started by adding oxalacetate.

RESULTS AND DISCUSSION

As shown in Fig. 1, α-ketoglutarate markedly inhibits citrate synthetase activity and seems apparently to compete with oxalacetate. The K_i value obtained by replotting the slopes of lines in Fig. 1 (See, inset) against various α-ketoglutarate

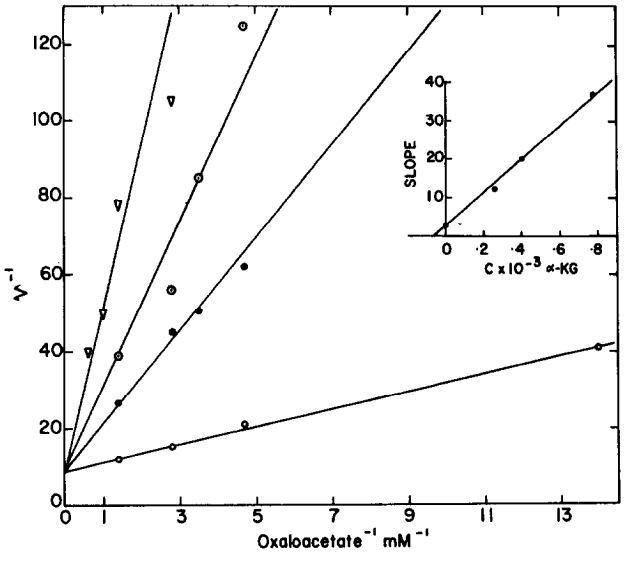

Fig. 1. The effect of α-ketoglutarate (α-KG) on the activity of citrate synthetase (pH 8.0) with oxalacetate as the variable substrate. The symbols are: O—O , no inhibitor; ●—● , 0.259 mM α-KG; ⊙—⊙ 0.389 mM α-KG; Δ—Δ 0.778 mM α-KG. One velocity unit is defined as an absorbancy increase of 0.001 at 412 mu per min. using the assay mixture described in the text.

concentrations is 6 x 10^{-5} M. The effect of α-ketoglutarate is specific. Other keto acids like pyruvate and phosphoenolpyruvate, and various metabolites related to the tricarboxylic acid cycle (glutamate, aspartate, isocitrate and α-ketovalerate), all tested at a concentration of 5 x 10^{-3} M do not affect enzyme activity.

Despite the apparent competitive inhibition (Fig. 1), the binding of α-ketoglutarate seems to occur at a site distinct from oxalacetate, as is borne out by the fact that some treatments which preserve the catalytic activity bring about a 'densensitization' of the enzyme to the inhibitory effect of α-ketoglutarate. Thus, at pH 8.0 the enzyme is inhibited to the extent of about 50% by 5 x 10^{-5} M α-ketoglutarate, but this inhibition is reduced to zero at pH 10.0 (Fig. 2). No inhibition is caused at this pH even at concentrations of α-ketoglutarate as high as 1.2 x 10^{-3} M

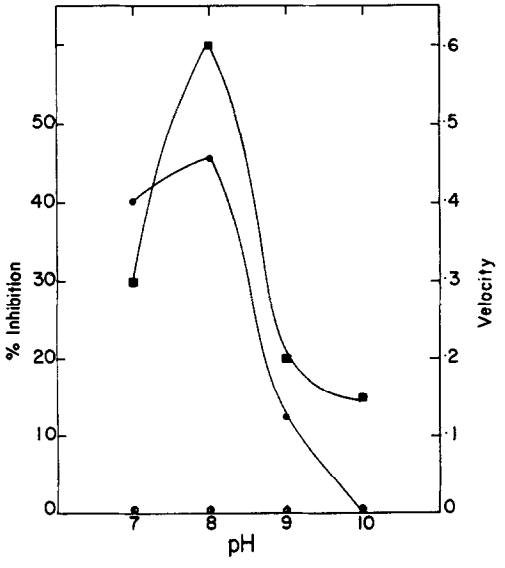

Fig. 2. The effect of pH on the activity of citrate synthetase in the absence or presence of α-KG. ■—■ , velocity as a function of pH; ●—● percent inhibition of activity in the presence of 0.40 mM α-KG; ○—○ percent inhibition by α-KG (0.4 mM) in the presence of 0.1 M KCl. In all assays, 0.02 M Tris, 0.04 mM acetylCoA and 0.100 mM oxalacetate was used.

(about 30 x K_i). Similarly, inclusion of 0.1 M KCl in the assay medium completely abolishes inhibition by α-ketoglutarate at all pH values tested (Fig. 2). As already demonstrated by Weitzman (1966) KCl causes some stimulation of enzyme activity at pH values above and below 8.0, but 'desensitization' to α-ketoglutarate does not seem to be connected with this stimulation, since at pH 8.0, 0.1 M KCl does not cause any alteration of enzyme activity, and α-ketoglutarate still does not inhibit the enzyme even at concentrations as high as 20 x K_i. The conclusion, therefore, seems inevitable that α-ketoglutarate serves as an allosteric inhibitor of the enzyme. This conclusion is strengthened by our finding (to be published in detail elsewhere) that α-ketoglutarate brings about complex changes in the kinetic behaviour of citrate synthetase. The initial velocity plots with acetylCoA as the variable substrate, linear in the absence of α-ketoglutarate, become non-linear in its presence.

The characteristics of inhibition of citrate synthetase by α-ketoglutarate are very similar to those described for $NADH_2$ (Weitzman, 1966) and palmitylCoA (Srere and Whissen, 1967). Like α-ketoglutarate, no inhibition by $NADH_2$ occurs at pH values above 9.0. Further, in the presence of 0.1 M KCl, the enzyme is 'desensitized' to the affect of $NADH_2$ and palmitylCoA. Owing to the structural dissimilarity of $NADH_2$ and palmitylCoA on one hand and α-ketoglutarate on the other, it does not seem likely that they share the same binding site. This is also borne out by the fact that in contrast to α-ketoglutarate, $NADH_2$ and palmitylCoA act as non-competitive inhibitors of oxalacetate.

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